

Mono Q chromatography permits recycling of DNA template and purification of RNA transcripts after T7 RNA polymerase reaction

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Biophysical and biochemical analysis of RNA often requires milligram amounts of pure RNA species. *In vitro* T7 RNA polymerase transcription of cloned genes encoding the desired RNA has proven immensely useful for obtaining large quantities of specific RNA species (1, 2). Separation of the desired RNA species is necessary after transcription in order to remove the DNA template and, in some cases, additional contaminating smaller RNA transcripts (3). Gel electrophoretic and chromatographic separation methods as well as DNase treatment have been employed (4, 5).

Here we present a fast and simple, one-step-method based on Mono Q chromatography. The RNA product is separated from the template DNA which can then be re-used for several new rounds of *in vitro* transcription by T7 RNA polymerase. This technique represents a further development of a method described for the purification of supercoiled plasmid DNA (6).

As an example we present the transcription of a tRNA^{Gln} gene which was cloned behind the T7 RNA polymerase promoter (7). Nucleic acid samples are analyzed on a 10% polyacrylamide gel. In order to generate the proper 3'-end of the transcription product (the tRNA CCA end) the plasmid DNA was cleaved with *Bst*NI (see three bands in Figure 1A, lane 1). A 2 ml transcription reaction (7) containing 250 µg DNA was extracted with phenol/chloroform and subsequently ethanol precipitated (Figure 1A, lane 2). The dried nucleic acid pellets were redissolved in Mono Q buffer (20 mM MOPS, pH 6.25, 350 mM NaCl) and directly applied to a preequilibrated Mono Q 5/5 column (1 ml) at a flow rate of 1 ml/min. The column was washed with 350 mM NaCl in Mono Q buffer to remove the excess nucleoside triphosphates (Figure 1B, peak N). A 10 ml gradient (350–750 mM NaCl) was used for the subsequent elution of the tRNA (Figure 1B, peak labelled RNA) and the DNA template (Figure 1B, peak labelled DNA). The material of both peaks was analyzed by gel electrophoresis (Figure 1A, lanes 3 and 4, respectively). The RNA samples were pure by sequence analysis and could also be successfully aminoacylated (7). As a test of the integrity of the recovered DNA another round of *in vitro* transcription was attempted after concentration of the sample by ethanol precipitation. As the analysis in Figure 1A, lane 5 shows the recovered DNA is a good template for transcription. Quantitation of the RNA yield showed that the template activity of the DNA was unchanged or better. The recovery of RNA and DNA applied to the Mono Q column was around 90%. Large amounts of nucleic acids can be applied to the Mono Q column (up to 2.5

mg nucleic acids per ml of column material) without loss of resolution. While phenol/chloroform extraction of the nucleic acids was routinely used before application of the material to the column we know that good separation also occurs if the total reaction mixture is applied immediately to the column.

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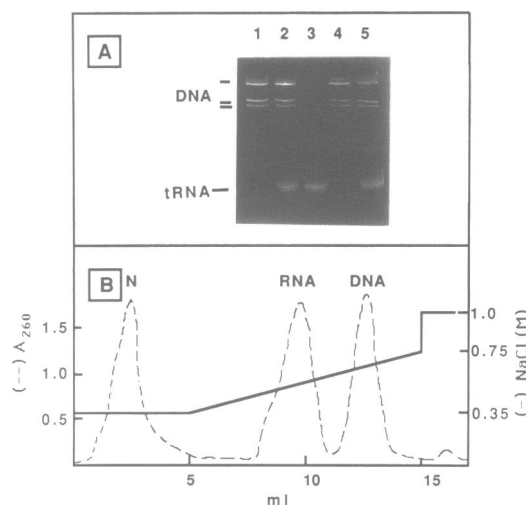


Figure 1. A, Electrophoretic separation of nucleic acid. Lane 1: 10% polyacrylamide gel of plasmid DNA after *Bst*NI cleavage. Lane 2: Cut plasmid DNA and RNA product after T7 transcription. Lane 3: Material from peak labelled RNA. Lane 4: Material from peak labelled DNA. Lane 5: T7 transcription of the DNA shown in lane 4. B, Elution profile of transcription reaction (lane 2 of panel A) chromatographed on Mono Q 5/5.